Research Circular 61 December 1958

SOIL NITROGEN COMPLEXES:

I. Chromatography of Amino Compounds in Soil Hydrolysates

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SOIL NITROGEN COMPLEXES: I. CHROMATOGRAPHY OF AMINO COMPOUNDS IN SOIL HYDROLYSATES

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SUMMARY

Chromatographic methods were employed to determine qualitative and quantitative differences in amino nitrogen compounds from soil organic matter of two virgin and four long-time rotation soils.

Hydrolysates were prepared for ion exchange and paper chromatography by autoclaving acid-soil mixtures and clarifying the crude hydrolysate by evaporation, centrifugation and desalting procedures.

The strong acid treatment of the soil samples released approximately 75 (71-83) percent of the total soil nitrogen in soluble form. Approximately one-third of the total soil nitrogen was hydrolyzed to ammonia and an additional one-third to α-amino nitrogen.

More than 50 ninhydrin reactive substances were detected in the desalted hydrolysates from some Ohio soils. Twenty-three of these constituents were identified: cysteic acid, glucosamine, aspartic acid, serine, threonine, glutamic acid, methionine sulfone, hydroxyproline, glycine, alanine, beta-alanine, γ-amino-butyric acid, valine, methione sulfoxide, proline, isoleucine, leucine, ornithine, lysine, histidine, tyrosine, arginine and phenylalanine. Four others were tentatively identified: galactosamine, α, ϵ-diaminopimelic acid, α-amino butyric acid, n-acetyl glucosamine. No qualitative differences were observed in the distribution of those amino compounds present in large amounts, even in soils having different cropping histories. Quantitative estimations were obtained indicating relative magnitudes of components identified.

The detection of so many ninhydrin reactive substances indicates an important advance in the use of ion exchange and paper chromatographic techniques as applied to soils. It is evident, however, that greater improvements are yet desirable particularly for quantitative separation and determination of the many substances appearing in small amounts.

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INTRODUCTION

Ion exchange and paper chromatography have been successfully used in recent years to study nitrogen complexes of the soil. Bremner, (1950) used two-directional paper chromatography to identify 22 amino acids and an amino sugar from soil hydrolysates. Stevenson (1956) employed a modified Stein & Moore (1949) ion exchange technique which resulted in an isolation of 33, and identification of 29 ninhydrin-positive compounds from acid hydrolysates of Illinois soils. Sowden (1955) reported isolation of 15 and identification of 14 ninhydrin-positive compounds. (See Table 4). All of these studies were made on solutions obtained by hydrolyzing soil organic matter or fractions thereof with varying amounts of 6N HCl. (This reagent does not give complete solution of the total soil nitrogen, but reportedly yields the maximum hydrolysis of the reagents so far employed, Bremner, 1949).

Until recent years attention has been given only to the 20-25 amino acids known to be naturally occurring constituents of protein. Now, more than 50 different amino acids have been found in nature. Since, no more complex mixture of naturally occurring organic materials exists than in soils, it seemed logical that more of the uncommon amino compounds might be found in soil systems. The purpose (though not always stated) of the studies previously mentioned, and of the one from which this report is taken, was to more completely characterize the chemical composition of the nitrogen complexes existing in soils. The intent of this paper is to report results of extensive and more exhaustive attempts to determine possible qualitative and quantitative differences in amino nitrogen compounds of soil organic matter by using chromatographic techniques.

MATERIALS AND METHODS

SOILS

Field samples of virgin Hoytville clay loam, virgin Wooster silt loam and Wooster silt loam from four long-time rotation plots were air-dried, sieved (∠2mm., undecomposed residues previously removed) and analyzed for organic matter (Walkley-Black, see Peech, **et al.** 1947), total nitrogen (micro-Kjeldahl), total carbon (Van Slyke, **et al.** 1940, 1951) and pH (glass electrode). Characteristics and treatments of the soils are shown in Table 1.

TABLE 1.—Description of Soils Studied

Soil	pH	Organic matter	Total carbon	Total nitrogen	C/N
		%	%	%	
Wooster silt loam*					
9(K)	6.6	1.5	0.74	0.079	9.4
10(K)	6.4	1.4	0.74	0.079	9.4
3(L)	6.7	1.4	0.75	0.081	9.1
5(L)	6.8	1.5	0.79	0.084	9.3
Virgin†	5.2	2.3	1.15	0.108	10.6
Hoytville clay loam					
Virgin‡	6.0	10.1	6.65	0.550	12.1

* 9(K) 1915-36 Continuous vetch
1937-53 Continuous corn
1954-55 Corn, wheat

10(K) 1915-36 Continuous timothy-bluegrass
1937-53 Continuous corn with sweetclover catch crop
1954-55 Corn, wheat

3(L) 1915-35 Corn, soybean rotation
1936-50 Corn, oats with sweetclover catch crop
1950-53 Corn, oats with 8 tons/acre manure on corn
1954-55 Corn

5(L) 1915-53 Corn, oats rotation
1954-55 Corn

All plots similarly fertilized.

†Virgin Wooster silt loam—Native sod: principally bluegrass.

‡Virgin Hoytville clay loam—Forest: Burr oak, red oak, ash, elm, shagbark hickory with some bluegrass.

PREPARATION OF HYDROLYSATE

The soils were hydrolyzed by autoclaving at 121° C. and 15 psi. for 18 hr. with 6N HCl (500 ml/400 g. air-dried soil). The autoclaved mixtures were filtered, the residues thoroughly washed with hot 1N HCl and distilled water. Excess HCl was removed from the filtrates (acid extract) by repeated **in vacuo** evaporation at 50° C. Following centrifugation to remove portions not soluble in 200 mls of water, desalting of the hydrolysate was accomplished by the procedure of Stevenson (1954) except that Dowex-50, 8% cross-link, 100-200 mesh resin was used instead of Amberlite IR-12OH. Convenience of supply dictated choice of the Dowex resin. Since both the Amberlite IR-12OH and Dowex-50 are strongly acidic resins, this variation was not expected to alter the desalting technique.

Recovery of nitrogen during these preparatory procedures was followed by determining total nitrogen (micro Kjeldahl, using bromcresol—new coccine—p-nitrophenol indicator, Sher, 1955), ammonia (Varner, **et al.** 1953) and ∝-amino nitrogen (Van Slyke, **et al.** 1941) in the soil residue from the acid extract and in the crude, centrifuged, and desalted hydrolysates. The indicator mentioned for the micro Kjeldahl procedure was found to give a better endpoint and more reproducible results than other commonly used indicators.

COLUMN CHROMATOGRAPHY

The resin and chromatography column were prepared by the same general technique as that described by Stevenson (1954) with the following modifications: (a) Dowex-50, 8% cross-link (∠270 mesh with 10% of the fines removed) resin in the hydrogen form was used, (b) a glass wool plug replaced the sintered glass support disk, (c) the resin bed was increased to 120 cm. in length, and (d) length of the delivery tip was reduced to a minimum in order to prevent mixing of separated components. Equilibration of the resin was accomplished by leaching the column with 0.5N HCl containing 0.25% BRIJ-35 detergent for 24 hours prior to use. The modifications permitted adequate flow rate of the eluting agent yet increased resolution by yielding more and sharper peaks than otherwise obtained. The detergent was found necessary for consistent and efficient operation of the measuring siphons.

An aliquot (in duplicate) of desalted hydrolysate containing 2-3 mg. of amino nitrogen was carefully added to the top of the column and washed into the resin bed with several minimal portions of distilled water. Proper sample placement was important—the more narrow and even the initial zone of absorption, the better the resolution. Chromatographic separation was accomplished by successive elution with a graded series of HCl solutions, 1.5N, 2N and 4N, containing 0.25% BRIJ-35 detergent. One ml. fractions were continuously collected with an Autonomas fraction collector at a flow rate of 4 ml. per hour. Attempts to increase the flow rate appreciably resulted in losses of both resolution and recovery. Concentration of ninhydrin reactive substances appearing in each fraction was determined on a suitable aliquot by the method of Troll and Cannan (1953). Proline was determined by the method of Chinard (1952) and hydroxyproline by the method of Troll and Cannan (1953) and Rogers, **et al.** (1954). From a plot of the mM concentration vs. the fraction tube number, the quantity of any ion exchange separated constituents was determinable by integration of the area under the peak.

IDENTIFICATION OF AMINO COMPOUNDS

A reference elution diagram was prepared by chromatographing a mixture of known amino acids by the procedure outlined above. (See Fig. 1).

Tubes of effluent from the column corresponding to a single peak on the elution diagram were combined. The excess acid was removed by evaporation at 50° C. and the residues were then dissolved in distilled water and redried **in vacuo.** Each residue should theoretically represent one pure compound as separated by the ion exchange column. Positive identification was accomplished by one-directional paper chromatography using three solvent systems and three color reagents. Varying quantities of freshly prepared aqueous solutions of the above residues were spotted on sheets of Whatman No. 1 paper together with spots of known amino acids. Triplicate papers were then placed in each of three solvent systems: phenol-water (75:25, W:W), butanol-acetic acid-water (40:10:50, V:V:V), and collidine-lutidine-water (100:100:100, V:V:V) (Saifer & Oreskes, 1956 and Block, **et al.,** 1952). Solvent fronts traveled at least 40 cm. beyond the base line with butanol-acetic acid-water and at least 15cm. with the other two solvent systems. Spots were developed on the first of the triplicate papers with ninhydrin, on the second with isatin and on the third with alloxan (Saifer and Oreskes, 1956). Identification was considered positive when the unknown matched a known compound in both color and R^f on all the three papers from all three solvent systems.

The same procedure was employed in the paper chromatography of residues from the soil hydrolysates. In addition, however, application of overloading quantities of the ion exchange separated residues to the filter paper sheets was required for separation and detection of many overlapping substances present in relatively small amount.

RESULTS AND DISCUSSION

PREPARATION OF HYDROLYSATES

Representative data illustrating the distribution and recovery of ammonia, α-amino, and total nitrogen during preparation of the hydrolysates are seen in Table 2. Of the total soil nitrogen, 71-83 percent was solubilized by autoclaving the acid-soil mixture (1.25:1, V/W). Approximately one-third (27-32%) was released as ammonia nitrogen and another one-third as α-amino nitrogen (33-37%). These

figures were similar to results obtained when hydrolysis was accomplished by refluxing methods using considerably larger quantities of acid (Bremner,1949; Kojima, 1947; Sowden, 1955; Stevenson, 1954; present authors' unpublished data). Slightly more of the total nitrogen was released as ammonia from the cultivated soils than from the virgin soils in agreement with Stevenson's data (1956).

TABLE 2.—Distribution and Recovery of Nitrogen During Preparation of Hydrolysate

	Wooster silt loam		Hoytville clay loam
	Virgin	10(K)	
	%	%	%
Total soil nitrogen			
Fraction hydrolyzed by 6N HCl (crude hydrolysate)	83	75	71
to ammonia N	30	32	27
to ∝-amino N	37	33	36
Crude hydrolysate			
Proportion as ammonia N	38	41	39
as ∝-amino N	47	41	55
Centrifuged hydrolysate			
Proportion as ammonia N	41	44	42
as ∝-amino N	47	42	57
Desalted hydrolysate			
Proportion as ammonia N	4	7	4
as ∝-amino N	81	73	76
Recovery from desalting column			
of ∝-amino N applied	79	79	75
of total N applied	48	52	45

The clarifying procedures removed salts and organic materials, which interfere with subsequent chromatography, and also increased the relative proportion of ∝-amino nitrogen in the hydrolysates. As seen in Table 2, the desalting column was the most effective means of purifying towards ∝-amino N.

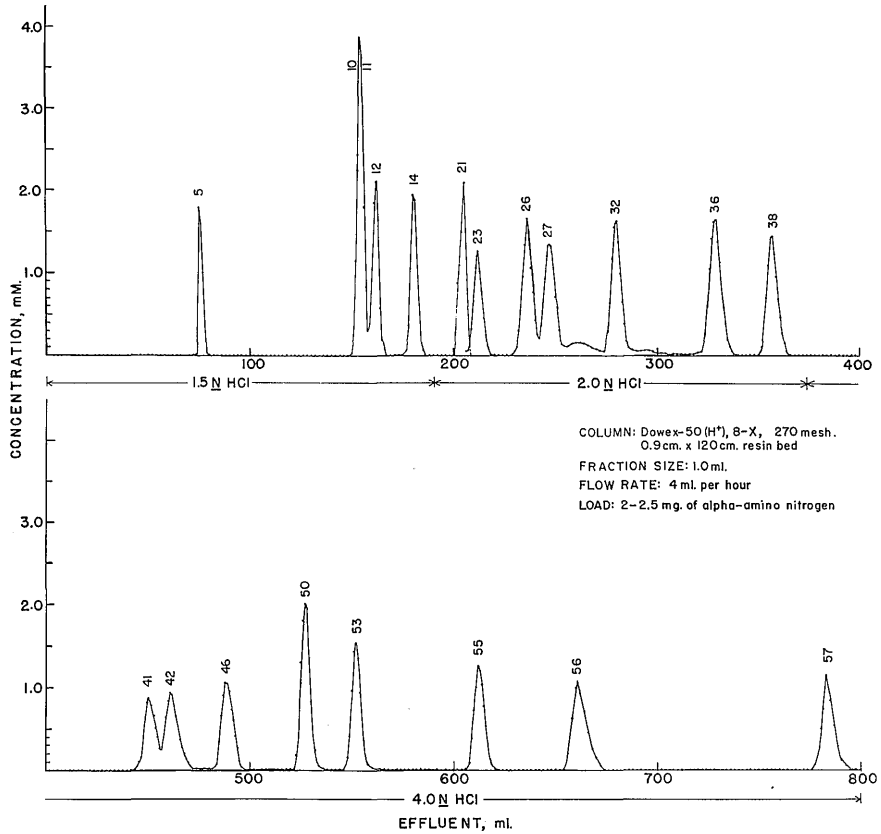

Fig. 1.—Elution diagram of a mixture of standard amino acids separated by ion exchange chromatography. The compounds identified by paper chromatography were: 5, unknown; 10, aspartic acid; 11, serine; 12, threonine; 14, glutamic acid; 21, hydroxy-proline; 23, glycine; 26, alanine; 27, beta-alanine; 32, gamma-amino butyric acid; 36, valine; 38, proline; 41, methionine; 42, isoleucine; 46, leucine; 50, lysine; 53, histidine; 55, tyrosine; 56, arginine; 57, phylalanine. Tryptophan and cysteine, present in the original mixture, were not recovered.

CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION OF AMINO COMPOUNDS

A reference elution diagram of a chromatographically separated mixture of pure amino compounds is shown in Figure 1. Identity of the compound present in each peak was established by paper chromatography as described under Materials and Methods.

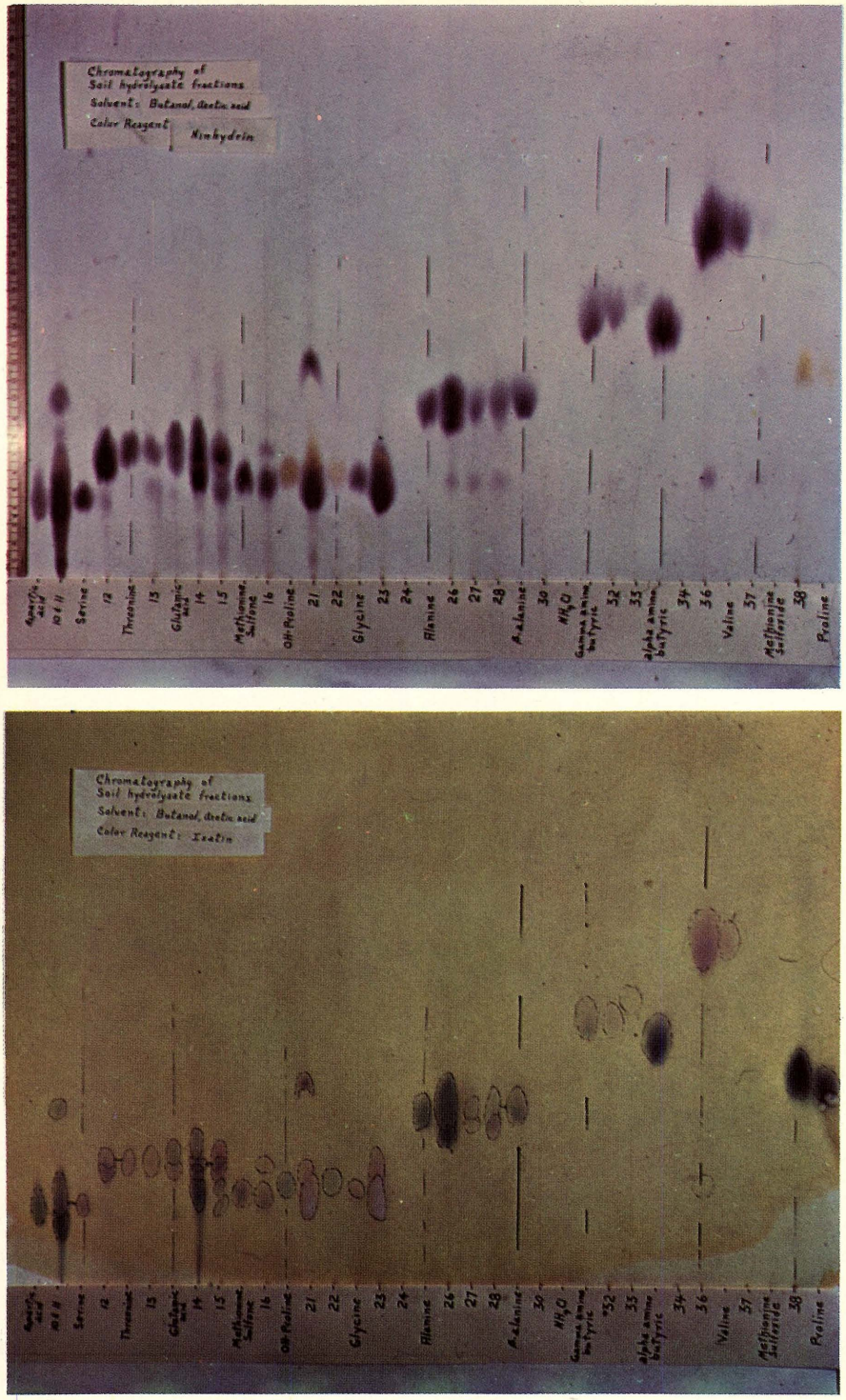

Plate 1. Color prints of ninhydrin (above) and isatin (below) developed chromatograms of known and unknown amino compounds, (peaks 10 to 38 of figure 2).

Plate 2. Color prints of ninhydrin (above) and isatin (below) developed chromatograms of known and unknown amino compounds, (peaks 2 and 39 to 57 of figure 2).

A representative elution diagram of the ion exchange chromatography of an aliquot of desalted soil hydrolysate is shown in Figure 2. Theoretically, peaks occupying the same relative position on Figures 1 and 2 should represent identical substances. Although this kind of comparison has often been used as evidence for positive identification of unknown compounds, it cannot be considered as conclusive proof, particularly when dealing with mixtures of unknowns of the complexity indicated in Figure 2. Numerous small peaks and some overlapping substances consistently appeared on the elution diagram of soil hydrolysates and were not attributable to errors in technique. Definitive spots were obtained on paper chromatograms from residues of many of these small peaks. Paper chromatography revealed the presence of several components in the unsymmetrical peaks. Even in symmetrical peaks, which are ordinarily indicative of pure compounds, more than one component was frequently encountered.

Color photographs of representative paper chromatograms using butanol-acetic acid-water solvent and ninhydrin and isatin color reagents are shown in Plates 1 and 2. The numerals along the base line correspond to residues from the numbered peaks on the elution diagrams of Figures 1 and 2. Known amino acids which were spotted on the same papers are listed by name. Many distinctive color reactions appeared on freshly developed chromatograms and were usually more reliable than R^f values when comparing known with unknown compounds for identification purposes. Some of the color differences remain clearly visible on Plates 1 & 2 in spite of inadequacies of color reproduction processes.

Only a few examples may be given here of the interesting results arising from comparisons of the paper chromatograms and corresponding elution diagrams. Isatin, of course, is not a specific reagent for detection of proline and hydroxy-proline contrary to previous information (Block, **et al.,** 1952). Although isatin is less sensitive than ninhydrin for many amino compounds, and although the colors which it develops are not stable, this reagent is superior to ninhydrin or alloxan because of the great variety of colors which are produced. Together with deliberate overloading of paper chromatograms, use of combinations of several solvent systems and color reagents made possible the detection of a large number of components not distinguishable with ninhydrin alone. In peak 14 (Plate 1) for example, at least four substances appeared with isatin in contrast to the one less distinctive ninhydrin spot. Peak 51, which commonly appeared as a slight dissymmetry on the trailing edge of the lysine peak, exhibited two closely associated components similarly distinguishable with isatin but not with ninhydrin.

Inadequacy of R^f values as identifying characteristics is emphasized. Peak 52-53 (Fig. 2) should have been histidine according to comparisons of reference elution diagrams and R^f values. As shown by the isatin color reaction (Plate 2) at least two additional substances having practically identical ion exchange and paper chromatography

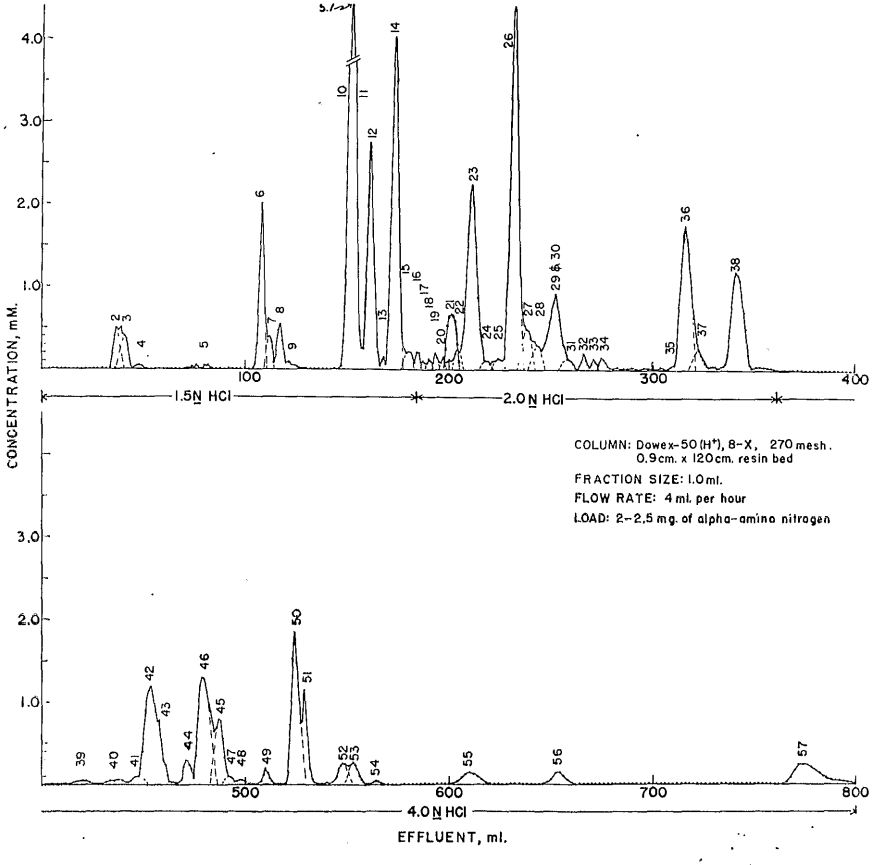

Fig. 2.—Ion exchange chromatography of ninhydrin reactive compounds from acid soil hydrolysates. Compounds in the numbered peaks identified by paper chromatography were: 2, cysteic acid; 4, n-acetyl glucosamine (?); 6, glucosamine; 10 and 11, aspartic acid and serine; 12, threonine; 14, glutamic acid; 15, methionine sulfone; 21, hydroxy-proline; 23, glycine; 26, alanine; 27, beta-alanine; 30, ammonia; 32, gamma-amino butyric acid; 36, valine; 37, methionine sulfoxide; 38, proline; 42, isoleucine; 46, leucine; 45, ornithine; 50, lysine; 53, histidine; 55, tyrosine; 56, arginine; 57, phenylalanine.

behavior were present besides histidine in the soil hydrolysates. Peak 41 (Fig. 2) correspond to methionine (Fig. 1). Several substances were detected in residues from this small peak and although R^f values were comparable in some instances, color reactions and other R^f values were sufficiently different from known methionine to rule out positive identification of this sulfur containing amino acid. The same was true of peak 44 which should correspond to cystine as identified by Stevenson (1956). Cysteic acid, another sulfur containing amino acid, was positively identified however (numeral 2, Plate 2), though it is reportedly lost during some desalting procedures (Bremner, 1950).

Ornithine (peak 45) was found to be a transient, i.e., its relative position on elution diagrams varied from the trailing edge of the leucine peak to the trailing edge of the isoleucine peak, a shift of 35 to 40 fractions. Though temperatures was a contributing factor the exact cause of this shift was not determined. Nevertheless, this mobility emphasizes the inadequacy of using comparable positions on elution diagrams as the criterion for identification of unknown substances.

In all, more than 50 distinct ninhydrin-reactive compounds were detected on the paper chromatograms of residues corresponding to the peaks (numbered through 57) on the elution diagrams from soil hydrolysates. Twenty-three of these were identified and are listed by name in Figure 2. Four more constituents were tentatively identified by their similarity of reaction to known compounds. These were n-acetyl glucosamine, galactosamine, α-amino-n-butyric acid, and α, ϵ-diaminopimelic acid (peaks 2, 7, 34 and 31 respectively, Fig. 2). Most of the unidentified constituents were collected in quantities too small to permit positive identification. The procedures employed may have created some artifacts, but it is apparent that acid hydrolysates of soil contain many more amino compounds than previously recognized.

DISTRIBUTION OF AMINO ACIDS IN SOME OHIO SOILS

The recovery of α-amino nitrogen added to the chromatography column was essentially complete even though isolation in a pure form of the many individual trace components was not achieved with the ion exchange column. Results indicated the qualitative distribution of amino compounds to be similar in all soils examined. Estimation of the relative amounts of amino compounds in Wooster silt loam and Hoytville clay loam soils (Table 3) was made on the assumption that the identified component comprised the major portion in that particular peak (Fig. 2). While the figures are not strictly quantitative because of incomplete separation, they certainly may be taken as indicative of the relative orders of magnitude. The Hoytville clay loam contained

TABLE 3.—Relative Amounts of Amino Acids in Some Ohio Soils

Peak No.	Amino Acid	Mg. per 100 g. of soil					
		9(K)	10(K)	3(L)	5(L)	V-W	V-H
2	Cysteic acid	5	5.5	5.5	3.5	5	11
10	Aspartic acid*	25	29	27	32	43	182
11	Serine*						
12	Threonine	12	11	10	13	16	80
14	Glutamic acid	22	22	23	26	33	137
15	Methionine sulfone	3	0.8	1.8	2.0	1.1	10
21	Hydroxyproline	3	4	3	5	4	33
23	Glycine	10	10	11	10	12	80
26	Alanine	14	17	17	18	22	109
27	Beta-alanine	2	1	2	2	2	10
32	Gamma-amino-butyric acid	0.4	0.3	0.5	0.3	0.7	1.7
36	Valine	11	11	11	11	16	81
37	Methionine sulfoxide	0.5	0.8	0.5	0.6	1.0	6.4
38	Proline	8	9	9	10	12	48
42	Isoleucine	6	7	7	8	9	33
46	Leucine	6	7	6	7	9	58
45	Ornithine	6	7	8	8	5	35
50	Lysine	11	13	13	13	17	69
52	Histidine	2	0.3	0.7	0.8	3.5	13
55	Tyrosine	0.6	1.0	____	Trace	1.5	17
56	Arginine	1	1	____	1	1	12
57	Phenylalanine	5	4	4	5	7	34

*From the spots on the paper chromatograms, these amino acids appeared to be present in roughly equivalent amounts.

considerably larger quantities (5-8x) of amino compounds than the Wooster silt loam soils (basis of oven-dry soil). However, calculated per unit weight of total soil carbon rather than unit weight of soil, the quantity of each amino compound present was approximately the same in all soils.

For comparative purposes Table 4 has been included to show the identified amino nitrogen compounds detected in acid hydrolysates of soil organic matter fractions as determined by different investigators. Figures for the representative soils indicate the relative abundance of the constituents listed. Of course, direct comparisons between investigators are not possible because the techniques were somewhat different in each case. However, the data indicate that the bulk of amino nitrogen present in soils can be accounted for by 12-15 compounds. Qualitatively the distribution of the more abundant amino compounds

TABLE 4.—Comparison of Identified Amino Nitrogen Compounds from Acid Hydrolysates of Soil Organic Matter Fractions. Isolations Reported by Different Investigators Using Ion Exchange and Paper Chromatographic Techniques.

	Relative Abundance of Amino Compounds			
	Bremner (1955)	Stevenson (1956b)	Sowden (1955)	Young & Mortensen
	Prep. 1P‖	Flanagan s.1.†	Lacombe soil‡	Hoytville c.1.§
	1=weak 10=strong	mg/100g soil	mg/100g soil	mg/100g soil
Cysteic acid	1	16	--	11
N-Acetyl glucosamine	--	+	--	P
Glucosamine	}	+	--	+
Galactosamine	} <1	+	--	P
Aspartic acid	8	67	146	}
Serine	6	73	48	} 182
Threonine	4	81	79	80
Glutamic acid	9	94	117	137
Methionine Sulfone	<1	+	--	10
Hydroxyproline	M	+	--	33
Glycine	9	74	81	80
Alanine	9	77	46	109
β-Alanine	2	17	--	10
γ-Aminobutyric acid	2	12	--	2
α, ε-Diaminopimelic acid	P	10	--	P
α-Amino-n-butyric acid	1	3	--	P
Valine	8	62	58	81
Methionine Sulfoxide	<1	23	--	6
Proline	M	52	40	48
Methionine	--	+	12	--
Isoleucine	}	48	34	33
Leucine	} 10	63	39	58
Ornithine	--	9	--	35
Cystine	--	+	+	--
Lysine	3	70	--	69
Histidine	<1	7	--	13
Tyrosine	1	23	26	17
Arginine	2	17	--	12
Phenylalanine	2	24	36	34

*Symbols in table:
M = medium.
P = provisional identification.
+ = constituent identified but quantity not estimated.
-- = not detected.

‖Clay loam from Rothamstead, England.

†Brunizem—Flanagan silt loam, under grass sod, Urbana, Illinois.

‡A black soil from Lacombe, Alberta, Canada.

§Clay loam from under deciduous forest, Hoytville, Ohio.

appears to be the same for all soils tested. Quantitatively there are indications that the proportion of some amino acids may vary considerably from one soil type to another.

CONCLUSION

The more refined the techniques employed in study of soil nitrogen, the more complex the system appears. The bulk of the amino-nitrogen present in soil hydrolysates may be accounted for by 12-15 amino acids. The majority of the ninhydrin-positive compounds detected in this study obviously must exist in minute quantities. The significance of the presence of any of the latter has yet to be determined. It would be presumptive and premature to conclude that because the amounts are minute their occurrence is inconsequential.

The detection of the large number of ninhydrin-reactive substances indicates an important advance in the use of ion exchange and paper chromatographic techniques as applied to soils. Refined instrumentation and careful interpretation may extend chromatographic techniques to yield quantitative results even for the substances existing in small amounts.

ACKNOWLEDGMENT

Appreciation is extended to F. J. Stevenson for suggestions and assistance by correspondence.

LITERATURE CITED

Block, R. J., LeStrange, R., and Zweig, G. 1952. **Paper Chromatography.** Academic Press Inc., New York.

Bremner, J. M. 1949. Studies on soil organic matter. Part I. The chemical nature of soil organic nitrogen. J. Agr. Sci. 38:183-193.

Bremner, J. M. 1950. The amino acid composition of the protein material in soil. Biochem. J. 47:538-542.

Chinard, F. P. 1952. Photometric estimation of proline and ornithine. J. Biol. Chem. 199:91-95.

Kojima, R. T. 1947. Soil organic nitrogen: I. Nature of the organic nitrogen in a muck soil from Geneva, New York. Soil Sci. 64:157-165.

Peech, M., Alexander, T., Dean, L. A., and Reed, J. F. 1947. Methods of soil analysis for soil fertility investigations. U.S.D.A. Circ. No. 757.

Rendig, V. V. 1951. Fractionation of soil nitrogen and factors affecting distribution. Soil Sci. 71:253-267.

Rogers, C. J., Kennel, J. R., Hutchins, M. E., and Harper, H. A. 1954. A hydroxy-proline method of analysis for a modified gelatin in plasma and urine. J. Biol. Chem. 206:553-559.

Saifer, A. and Oreskes, I. 1956. Color reaction of amino acids with alloxan, isatin and ninhydrin in circular paper chromatography. Anal. Chem. 28:501-504.

Sher, I. H. 1955. Two-step mixed indicator for Kjeldahl nitrogen titration. Anal. Chem. 27:831-832.

Sowden, F. J. 1955. Estimation of amino acids in soil hydrolysates by the Moore and Stein method. Soil Sci. 80:180-188.

Stein, W. H. and Moore, S. 1949. Chromatographic determination of the amino acid composition of proteins. Cold Spring Harbor Symposia Quant. Biol. 14:179.

Stevenson, F. J. 1954. Ion exchange chromatography of the amino acids in soil hydrolysates. Soil Sci. Soc. Amer. Proc. 18:373-377.

Stevenson, F. J. 1956a. Isolation and identification of some amino compounds in soils. Soil Sci. Soc. Amer. Proc. 20:201-204.

Stevenson, F. J. 1956b. Effect of some long-time rotations on the amino acid composition of soil. Soil Sci. Soc. Amer. Proc. 20:204-208.

Troll, W. and Cannan, R. K. 1953. A modified photometric ninhydrin method for the analysis of amino and imino acids. J. Biol. Chem. 200:803-811.

Van Slyke, D. D. and Folch, J. 1940. Manometric carbon determination. J. Biol. Chem. 136:509-541.

Van Slyke, D. D., Dillon, R. T., MacFadden, D. A., and Hamilton, P. 1941. Gasometric determination of carboxyl groups in free amino acids. J. Biol. Chem. 141:627-669.

Van Slyke, D. D., Plazin, J., and Weisiger, J. R. 1951. Reagents for the Van Slyke-Folch wet carbon combustion. J. Biol. Chem. 191:299-304.

Varner, J. E., Bulen, W. A., Vanecko, S. and Burrell, R. C. 1953. Determination of ammonium, amide, nitrite, and nitrate nitrogen in plant extracts. Anal. Chem. 25:1528-1529.